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Purification of Measles Virus and Characterization of Subviral Components

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Purified measles virus was obtained from [^{35}S]methionine-labeled cells infected at 33°C and maintained in the absence of fetal calf serum. The pellet that was produced by a single high-speed ultracentrifuge spin of culture medium contained virus of purity sufficient for structural analysis. Purified virions contain seven polypeptides with estimated molecular weights of: L, 200,000; G, 80,000; P2, 70,000; NP, 60,000; A, 43,000; F_1, 41,000; and M, 37,000, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions. Treatment of virions with 0.25% trypsin resulted in a less dense particle which lacked polypeptides G and F_1. Solubilization of the viral membrane with the detergent Triton X-100 in low-salt buffer resulted in the loss of the G polypeptide, whereas in the presence of 1 M KCl, Triton X-100 also removed most of the M polypeptide. The nucleocapsids (ρ = 1.3) obtained from virions treated with Triton X-100 and 1 M KCl contained the L, P2, NP, and M polypeptides. Nucleocapsids isolated from the cytoplasm of infected cells were predominantly composed of the NP polypeptide with smaller amounts of either polypeptide P2 or novel polypeptides, related to NP, with estimated molecular weights of 56,000 to 58,000 and 45,000 to 46,000. A significant amount of polypeptide L was always found in association with nucleocapsids isolated either from virions or from the cytoplasm of infected cells. A membrane component containing the viral membrane polypeptides G, F_1, and M was also isolated from infected cells. The data presented here thus suggest that L is an integral part of the nucleocapsid complex. In addition, 37,000-molecular-weight polypeptide (M) appears to have the function described for the matrix proteins of other paramyxoviruses.

Measles virus has been shown to be involved in the slowly progressive neurological disease subacute sclerosing panencephalitis (8, 11, 16, 38) and has been suggested by some workers to have a role in multiple sclerosis (4, 9, 18). The association of measles with these chronic diseases has resulted in increased interest in this virus and in tissue culture systems of persistent measles infection. The nature of the measles virus-host cell relationship in persistent infections is poorly understood. The elucidation of the specific mechanism(s) of initiation and maintenance of viral persistence will most likely require detailed knowledge of the wild-type virus and its overall strategy of replication. Studies on the structure and replication of measles virus have progressed significantly in the last few years (10, 22, 32, 34-36). However, certain difficulties continue to slow progress. One major problem has been the lack of reliable, efficient, and uncomplicated methods of virion purification (22, 32, 36). Thus, although recent work has defined the major polypeptides of measles virus, the structural relationships of the virion polypeptides, as well as the source and roles of minor bands (i.e., viral or cell), remain unclear (10, 22, 33, 37).

Measles is a paramyxovirus with a helical, RNA-containing nucleocapsid surrounded by a lipid- and glycoprotein-containing envelope. The first published characterization of the protein composition of measles virus reported the presence of six polypeptides, one that was associated with the nucleocapsid and two that were glycosylated and located exterior to the host-derived lipid envelope (12, 13). More recently, several laboratories have agreed that there are five virus-specific polypeptides, found both in the cytoplasm of infected cells and in purified virions. The polypeptides and their estimated molecular weights are: G, a glycoprotein, 80,000; P2, 70,000; NP, a component of the nucleocapsid, 60,000; P5, recently renamed F_1, 41,000; and M, 37,000 (10, 22, 32, 36). In addition, a protein of molecular weight 43,000 is found in virions, but has been identified as cellular actin (32, 33). A high-

molecular-weight polypeptide, L (150,000 to 200,000), has been identified in extracts from infected cells (36), but lack of resolution and varying amounts of the polypeptide have made it difficult to determine whether this polypeptide is specified by the viral or host genome. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of virions, [^{35}S]methionine-labeled material is often found near the dye front. This low-molecular-weight material may represent the F_2 polypeptide (10, 14, 22, 29), which, under nonreducing conditions, may be linked to the F_1 polypeptide.

This report describes a simple and effective first step in the purification of measles virions. Using this method, it was possible to isolate and compare nucleocapsids both from virions and from infected cells. Virion-derived nucleocapsids were composed of the L, P2, and NP polypeptides with varying amounts of M. Nucleocapsids obtained from the cytoplasm of infected cells contained primarily the L and NP polypeptides, with varying amounts of P2 and M. A number of "novel polypeptides" were often found associated with the L-NP protein complex isolated from infected cells. The location of the G and F_1 polypeptides has been confirmed as being on the outside of the virion envelope. Finally, the 37,000-molecular-weight virion polypeptide (the so-called M protein) was found to be associated with the internal portion of the virion envelope and appeared to have very strong interactions with the polypeptides of the nucleocapsid. These results confirm that M, whose function was previously assigned only by analogy to other viruses, is indeed the matrix protein of measles virus.

MATERIALS AND METHODS

Cell lines. CV-1 (African green monkey kidney) cells were grown as monolayers in tissue culture vessels in IMEMZO (Richter's improved minimal essential medium containing zinc, insulin, and 10 mM HEPES buffer [*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid], International Biological Laboratories), supplemented with 10% fetal calf serum.

Virus. The Edmonston strain of measles virus was used for all experiments. Viral stocks were prepared as previously described (36).

Isotopic labeling of intracellular viral proteins. When intracellular measles proteins were used as references, they were processed as described by Wechsler and Fields (36).

Purification of measles virus. Confluent tissue culture flasks (150-cm^2) were infected with virus at a multiplicity of infection of 1. After an adsorption period of 2 h at 33°C, 50 ml of IMEMZO containing 10% fetal calf serum was added to the infected cells, and incubation was continued at 33°C for 26 to 30 h. The serum-containing medium was then decanted, and cells were washed once with serum-free medium and incubated in 10 ml of methionine-free Eagle minimal essential medium containing 0.2 to 0.3 mCi of [^{35}S]-methionine (Amersham/Searle; specific activity, >400 mCi/mmol). Incubation, at 33°C, in this radiolabeled, serum-free medium continued until approximately 50 h postinfection, at which time the infected monolayer showed 80 to 100% cell fusion, with the multinucleate giant cells still attached to the plastic.

Virus was harvested by collecting the medium and clarifying it of cell debris by centrifugation at 1,000 × *g* for 30 min. The clarified medium was pelleted at 24,000 rpm in an SW27.1 rotor (Beckman) for 2 h. In most cases, this pellet was resuspended in the buffer appropriate to the projected experiment, or in gel sample buffer (17). For gradient centrifugation, the viral pellet was suspended in phosphate-buffered saline (PBS), layered onto a 15 to 50% (wt/vol) linear potassium tartrate gradient, and centrifuged overnight at 24,000 rpm in an SW27.1 rotor. The gradient was collected into 30-drop fractions, and a portion of each fraction was assayed for radioactivity by counting in toluene–Triton-based scintillation fluid in a Beckman LS-233 scintillation counter. Fractions showing radioactive peaks were pooled, diluted with PBS, and centrifuged for 2 h at 24,000 rpm in the SW27.1 rotor. The pellets were then suspended in gel sample buffer.

Preparation of infected cell extracts. Cells were infected, incubated, and labeled as described for virus purification. When cytopathic effect involved 80 to 100% of the cell monolayer (48 to 52 h postinfection), the medium containing labeled, released virions was decanted from 150-cm^2 tissue culture flasks and replaced by 10 ml of PBS containing 0.5 mM EDTA. The multinuclear "cells" were then incubated at 25°C until shaking detached the monolayer. The suspension was vigorously pipetted up and down, and then centrifuged at 1,000 × *g* for 30 min to pellet all large material. The 1,000 × *g* pellet, the source of "cytoplasmic nucleocapsid," was resuspended in 0.5 to 1 ml of distilled water, Dounce homogenized, and made 10% with concentrated Triton X-100 (Sigma). The detergent was dispersed with additional strokes of the homogenizer, and the nuclei were then removed by a 2-min centrifugation at 1,000 × *g*. The nuclei-free supernatant was layered onto a discontinuous CsCl step gradient (40%, 30%, 20%, wt/wt, in distilled water) with a 5% sucrose layer on top (7) and centrifuged for 16 to 18 h at 24,000 rpm in a Beckman SW27.1 rotor. The gradient was collected and assayed for radioactivity as described in the previous section. Radioactive peaks were collected by pelleting at 34,000 rpm for 90 min in a Beckman SW41 rotor. Alternatively, fractions containing radioactive peaks were diluted and recentrifuged onto a second identical gradient.

Trypsin treatment of purified virions. The method used was similar to that of Tyrrell and Norrby (32): 24,000-rpm pellets of released virus were suspended in PBS, PBS containing 0.1% trypsin (Sigma), or 0.25% trypsin (Grand Island Biological Co.). Digestion was for 1 h at 37°C. After treatment, virions were layered onto a 15 to 50% (wt/vol) potassium tartrate gradient and centrifuged for 16 to 18 h at 24,000 rpm in a Beckman SW27.1 rotor. The gradient was fractionated and assayed, and peaks were collected as described above.

Detergent treatment of virions. Virus pelleted from medium by centrifugation at 24,000 rpm was suspended in high-salt buffer (1 M KCl in 0.01 M phosphate buffer, pH 7.2) or low-salt buffer (0.01 M Tris buffer, pH 7.4). To each of the resulting suspensions 0.1 volume of concentrated Triton X-100 was added, and the mixtures were stirred for 1 h at room temperature. The samples were centrifuged at 11,000 rpm for 20 min in a Sorvall RC5 centrifuge. The resulting supernatants, representing soluble components, were processed as described in Results. The crude virion nucleocapsids (11,000-rpm pellets of Triton-insoluble material) were suspended in 0.1 M Tris buffer (pH 7.4) containing 1% Nonidet P-40 (Particle Data Laboratories, Ltd.) and 0.5 M urea and were layered onto a discontinuous CsCl gradient as described above. Centrifugation was for 16 to 18 h at 24,000 rpm in a Beckman SW27.1 rotor. Fractionation, assay, and collection of the gradient were as described above.

PAGE. SDS-PAGE of proteins was by the method of Laemmli (17). Electrophoresis was through a 10% polyacrylamide slab gel at a constant current of 45 mA for 2 to 3 h. Gels were stained, when applicable, in 50% methanol–7% acetic acid containing 0.2% Coomassie brilliant blue for 30 min. Electrophoretic destaining was for 30 min in 7% methanol–5% acetic acid. Gels were dried under vacuum and exposed to autoradiography with Kodak NS2T Safety Screen X-ray film.

Partial protease digests. Partial protease digests were performed by the method of Cleveland et al. (5), using 3 μg of *Staphlococcus aureus* protease V8 (Miles Laboratories) per sample.

RESULTS

Purification of measles virus. We have devised a simple technique that, in the initial stages of virion purification, avoids loss of virus as well as contamination with most cellular components. The method is described in detail in Material and Methods. Briefly, confluent cultures of CV-1 cells were infected with virus at a multiplicity of infection of 1 and incubated in serum-containing medium for 26 to 30 h at 33°C. At this time the medium was replaced with serum-free medium containing 0.2 to 0.3 mCi of [^{35}S]methionine, and incubation was continued at 33°C for another 18 to 20 h. Virus was harvested from the clarified culture medium by centrifugation at 24,000 rpm for 2 h in a Beckman SW27.1 rotor. In some cases the resulting viral pellet was taken up in PBS and subjected to density gradient centrifugation.

A comparison of the SDS-PAGE patterns of the polypeptides found in infected CV-1 cells and the polypeptides of virions purified in two different ways is shown in Fig. 1. The polypeptides present in a 24,000-rpm pellet suspended directly in sample buffer are shown in Fig. 1e. Lane f shows the polypeptides present in an identical viral pellet that was subjected to further purification by centrifugation in a potassium tartrate gradient. With the exception of a band migrating slower than L, the SDS-PAGE polypeptide pattern in these two preparations (Fig. 1e and f) is virtually identical, indicating that the initial step, prior to density gradient purification, provided considerable enrichment for, and partial purification of, radiolabeled virions. The polypeptide composition of the pelleted virions was similar to that reported by several laboratories (10, 22, 32, 36). Of particular

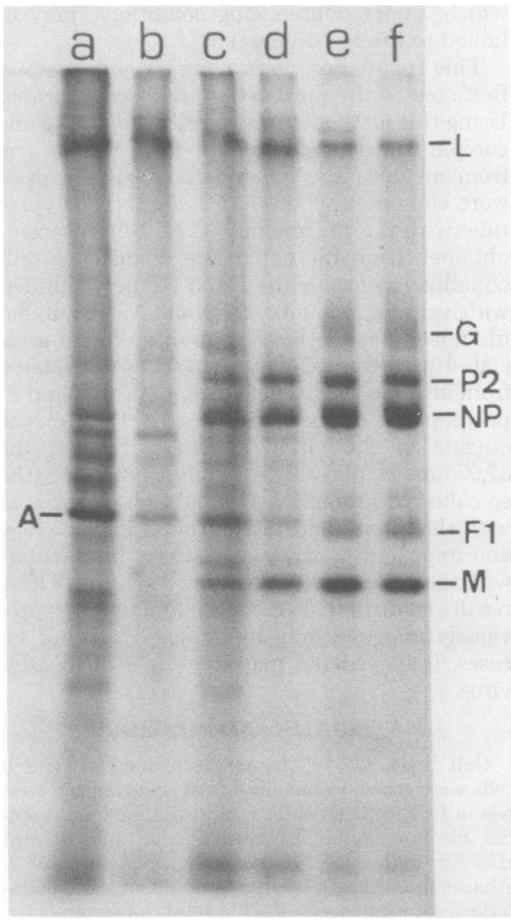

FIG. 1. *Polypeptides of control CV-1 cells, measles-infected cells, and released measles virions. Normal CV-1 cells and CV-1 cells infected with measles virus were grown, infected, radiolabeled with [^{35}S]methionine, and harvested as described in the text. Uninfected cytoplasmic (a) and nuclear (b) fractions, as well as infected cytoplasms (c) and nuclei (d), are included as markers. Virus was harvested from the medium over infected cells as a 24,000-rpm pellet (e), or (f) was subjected to further purification by equilibrium centrifugation in a linear potassium tartrate gradient (see Fig. 2). All samples were processed for SDS-PAGE and autoradiography as described in the text. Migration in this and subsequent gels is from top to bottom. Electrophoresis was for 2 h.*

interest was the appearance of the G glycoprotein. G was found in both cytoplasmic extracts of infected cells (Fig. 1c) and in released virions (Fig. 1e and f), but migrated differently in each case. The broad, fuzzy G band found in released virions is consistent with a highly glycosylated (or heterogeneously glycosylated) polypeptide, whereas the cellular version of this polypeptide migrated slightly faster, as a tight band. NP, P2, and M were the major labeled polypeptides in the pelleted preparation. The faint band near the dye front in Fig. 1e and f and Fig. 3c and d may be the F_2 band previously described (14), although we have no direct evidence for this point. The radioactive profile of the potassium tartrate gradient (Fig. 2) shows that the majority of the [^{35}S]methionine-labeled material contained in the 24,000-rpm pellet banded as a single peak ($\rho \simeq 1.18$), further evidence of the extent of purity of the 24,000-rpm pellet.

The success of this method was dependent upon two critical factors: temperature of virus growth and the presence or absence of serum during the period of radioactive labeling. The virus grown at 33°C is of higher titer than that

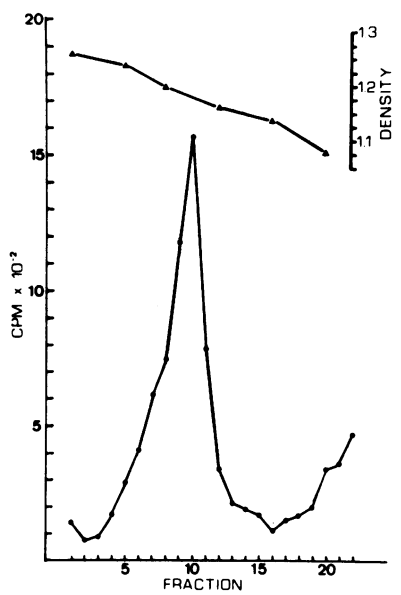

FIG. 2. *Analysis of measles virions by potassium tartrate gradient centrifugation. A 24,000-rpm virus pellet (described in Fig. 1) was layered on a linear 15 to 50% potassium tartrate gradient and centrifuged to equilibrium at 24,000 rpm in an SW27.1 rotor (Beckman). After centrifugation, the gradient was collected from the bottom into 30-drop fractions. Sedimentation was from right to left. A portion of each fraction was assayed for radioactivity as described in the text. The density of each fraction was determined by refractive index. The density of the virus is approximately 1.18.*

produced at 37°C (unpublished data). Virions grown at 33°C and isolated from the medium by pelleting at 24,000 rpm retained much of their infectivity. Plaque assays done on such virus pellets showed that 20% of the originally harvested infectivity was retained. Centrifugation onto a 60% sucrose cushion resulted in the retention of 63% of the starting infectivity. Thus measles virus grown at 33°C is structurally and biologically stable throughout the centrifugation used in the isolation procedure.

The presence of serum during the final 20 h of viral growth resulted in a heavy, unlabeled polypeptide band that migrated on SDS-PAGE in the vicinity of the P2 and NP viral polypeptides and caused difficulty with overloading of the gel. SDS-PAGE of 24,000-rpm pellets of virus grown in the presence or absence of serum is shown in Fig. 3. The heavy, serum-derived band is clearly seen in the region of P2 on the Coomassie blue-stained gel (Fig. 3b). The autoradiogram of the same gel (Fig. 3d) demonstrates the distortion caused by this unlabeled polypeptide that is not seen in viral preparations obtained from serum-free medium (Fig. 3a and c). Several polypeptide bands migrating between NP and A were found in the pellet shown in Fig. 3c. Polypeptides migrating in this region were occasionally seen in small amounts and may represent either breakdown products of viral polypeptides or contaminants present in the pellets.

Location of polypeptides in virions. i. Digestion of virions with trypsin. Trypsin digestion of intact virions is expected to remove all polypeptides external to the host-derived lipid bilayer. Polypeptides enclosed within the envelope, or imbedded within it, should be protected from the action of the protease (3, 25, 30). In this manner, polypeptides can be assigned internal or external locations in the virion.

The G polypeptide was removed when virions were treated with 0.1 or 0.25% trypsin at 37°C for 1 h (Fig. 4a and b). The F_1 polypeptide was resistant to digestion by 0.1% trypsin (Fig. 4b), as previously reported (32), but was removed by 0.25% trypsin (Fig. 4a). Virons incubated at 37°C in PBS were stable (Fig. 4c). When the digested virions were subjected to potassium tartrate density gradient centrifugation, they were found to be less dense than control virions (K. Stallcup, unpublished data), consistent with removal of outer glycoproteins (27).

The L, P2, NP, and M virion polypeptides are not susceptible to trypsin degradation, implying that these polypeptides are protected by (inside of) the lipid bilayer of the virion. With some enveloped viruses, protease treatment yields small hydrophobic tail fragments of external polypeptides (25, 30). Lanes a and b of Fig. 4

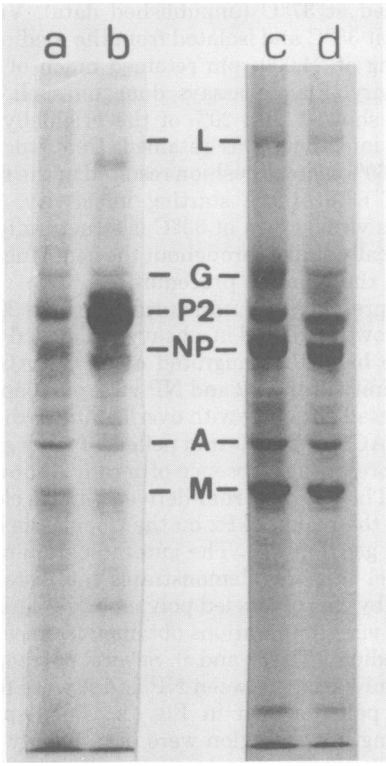

FIG. 3. *SDS-PAGE of the polypeptides of measles virions grown in the presence or absence of fetal calf serum. Two confluent monolayers of CV-1 cells were infected with virus at a multiplicity of infection of 1. At 30 h postinfection, one infected monolayer was washed with serum-free medium, and incubation was resumed in 10 ml of serum-free medium containing [^{35}S]methionine. The second infected monolayer was treated in an identical manner, except that the final labeling medium contained 10% serum. At 50 h postinfection, virus was harvested from the culture medium by centrifugation as described in the text. The viral pellets were subjected to SDS-PAGE. The gels were stained, destained, and processed for autoradiography as described in the text. (a) Stained gel of virus grown in serum-free medium; (b) stained gel of virus grown in the presence of serum; (c) autoradiogram of (a); (d) autoradiogram of (b). Electrophoresis was for 2 h.*

contain several new polypeptide bands, possibly digestion products representing such fragments.

ii. Isolation of measles nucleocapsid. Two methods were used to isolate nucleocapsid complexes from purified virions. The nucleocapsid preparation ($\rho \simeq 1.3$ g/cm^3) obtained by Triton X-100 treatment in low-salt buffer followed by CsCl density gradient centrifugation is shown in Fig. 5c. This nucleocapsid preparation contained L, P2, NP, and M polypeptides with some minor bands in the 43,000- to 46,000-dalton range (possibly breakdown products). The M polypeptide was present in significant quantities.

In contrast, nucleocapsids ($\rho \simeq 1.3$ g/cm^3) obtained by detergent extraction under high-salt

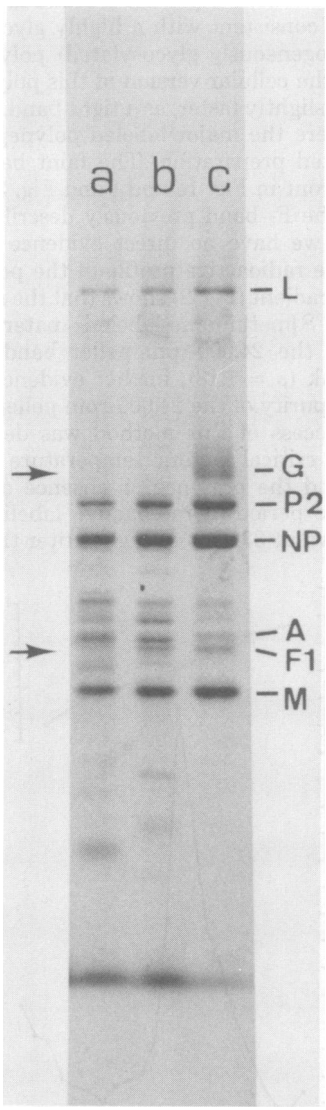

FIG. 4. *Removal of external virion polypeptides by trypsin digestion of measles virus. [^{35}S]methionine-labeled virus was prepared as described in the text. Identical samples were suspended in buffer containing various amounts of trypsin and incubated for 1 h at 37°C. Each sample was then layered onto a 15 to 50% linear potassium tartrate gradient and centrifuged for 16 h at 24,000 rpm in a Beckman SW27.1 rotor. Gradients were fractionated and counted, and the peak fractions were pooled and pelleted at 34,000 rpm for 90 min in an SW41 rotor. The pellets were processed for electrophoresis and autoradiography. (a) 0.25% trypsin; (b) 0.1% trypsin; (c) no trypsin. Electrophoresis was for 2.5 h.*

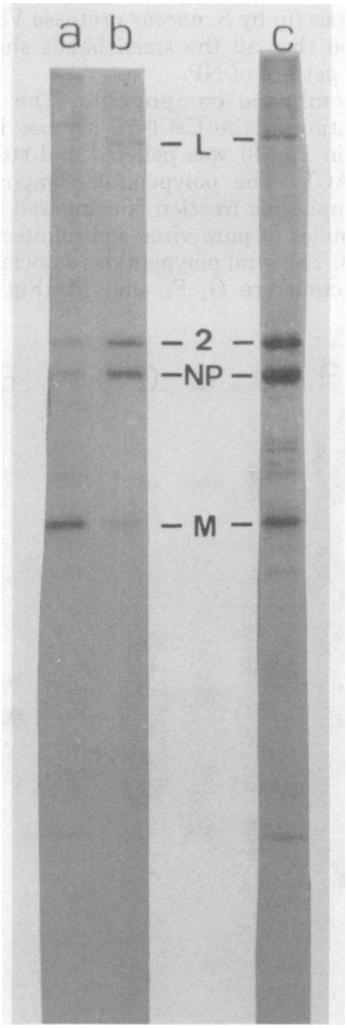

FIG. 5. *Polypeptide composition of nucleocapsids isolated from virions by detergent treatment. [^{35}S]-methionine-labeled measles virus was suspended in either high-salt (1 M KCl) or low-salt (0.01 M Tris) buffer. Triton X-100 was added to a final concentration of 10%, and the samples were incubated at 25°C for 1 h. Crude nucleocapsids were pelleted at 11,000 rpm for 30 min and further purified by equilibrium centrifugation in CsCl as described in the text. Peak fractions were pooled, pelleted, and processed for SDS-PAGE and autoradiography. The material solubilized by the high-salt and Triton X-100 treatment (11,000-rpm supernatant) was diluted with PBS and pelleted at 24,000 rpm for 2.5 h in an SW27.1 rotor. (a) High-salt supernatant; (b) high-salt nucleocapsid; (c) low-salt nucleocapsid. Electrophoresis was for 2 h.*

conditions (1 M KCl) followed by CsCl centrifugation showed significantly less M polypeptide in the final complex (Fig. 5b). The P2 and NP polypeptides were again major components, and the L polypeptide was found in small but significant quantities. Detergent extraction in the presence of 1 M KCl (as described in Materials and Methods) solubilizes the M polypeptide. To collect the M polypeptide, the solubilized material was diluted 10-fold with PBS and centrifuged for 2 h at 24,000 rpm in an SW27.1 rotor (Beckman). The resulting high-speed pellet was suspended in Laemmli gel sample buffer and subjected to electrophoresis (Fig. 5a). The pellet showed considerable enrichment of the M protein and contained residual amounts of polypeptides NP and P2, material in the molecular weight range of 43,000, but no L polypeptide. It is not clear whether the formation of the M pellet was due to precipitation after dilution of the KCl or whether the polypeptide was present in a sedimentable complex, as has been described for Sendai virus (15).

Isolation of viral polypeptides from infected cells. i. Nucleocapsids. "Cytoplasmic nucleocapsids" were obtained from homogenates of infected cytoplasm that were prepared as described in Materials and Methods and were layered on a CsCl–5% sucrose step gradient. The radioactive profile of the gradient (Fig. 6) reveals two peaks of sedimentable counts, one at a density of 1.3 g/cm^3 (fractions 9 and 10) and one at the CsCl–5% sucrose interface (indicated by the arrow, Fig. 6). An aliquot of pooled fractions 9 and 10, which was diluted and subjected to a second gradient purification, gave a single peak at density 1.3 g/cm^3 (data not shown). On examination by electron microscopy, the negative stain of pooled fractions 9 and 10 showed typical rough nucleocapsid complexes with subunits arranged in both linear, herringbone structures and disk-like monomers.

The analysis of this cellular nucleocapsid by SDS-PAGE is seen in Fig. 7c. The nucleocapsid preparation after a second density gradient purification is shown in Fig. 7d. Both preparations clearly contain the L polypeptide and another polypeptide that migrates ahead of L. Several polypeptides, including G, P2, and M, are present in minor amounts in both preparations, while the major species is clearly the NP polypeptide. In addition to the previously described viral polypeptides, three other major polypeptides (x, y, z), migrating between NP and A, are present in Fig. 7d. Nucleocapsids extracted by identical methods often yielded the structures shown in Fig. 7e, which contained polypeptides L, P2, and NP and small amounts of M. The nucleocapsids shown in Fig. 7e lack polypeptides x, y, and z and have a polypeptide composition that is virtually identical to the intact nucleocapsids obtained from virions (Fig. 5b and c).

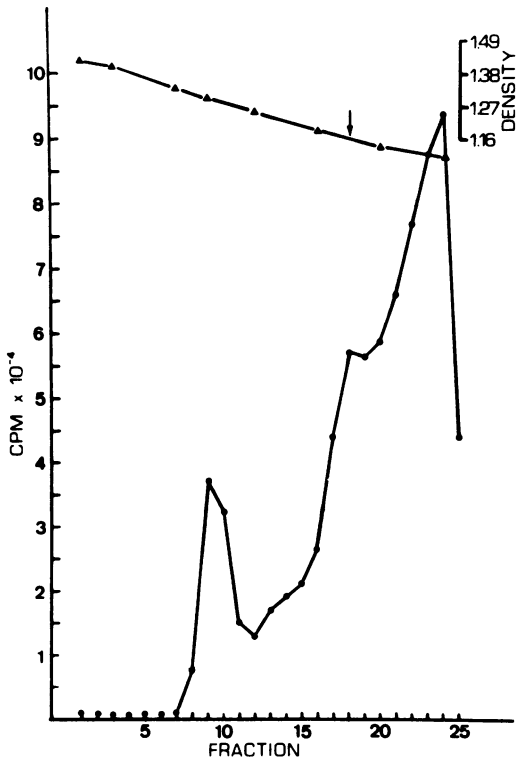

FIG. 6. *Analysis of [^{35}S]methionine-labeled cytoplasmic nucleocapsids on a CsCl step gradient. Monolayers of CV-1 cells were infected, labeled, and suspended in PBS-EDTA. Large cellular material was pelleted at 1,000 × g for 30 min and used as the source of cytoplasmic nucleocapsid. A homogenate of the 1,000 × g pellet was freed of nuclei, layered onto a 40%, 30%, 20% CsCl–5% sucrose step gradient, and centrifuged to equilibrium at 24,000 rpm in an SW27.1 rotor. The direction of centrifugation was from right to left. The gradient was collected from the bottom into 30-drop fractions, and a portion of each fraction was assayed for radioactivity as described in the text. Fractions containing peaks of radioactivity were pooled. A sample of pooled fractions 9 and 10 (ρ = 1.3 g/cm^3) was recentrifuged under identical conditions and gave a single radioactive peak of the same density.*

The novel polypeptides termed x, y, and z in Fig. 7c and d always appeared as homogeneous species, giving tight bands with characteristic migration during SDS-PAGE. Since the novel polypeptides were frequently found in nucleocapsids isolated from cytoplasm, and since there were previous reports of nucleocapsid breakdown (22, 24, 35), it was of interest to determine if x, y, or z were related to larger measles polypeptides. NP was obtained from gels of virion polypeptides, and bands x, y, and z were obtained from a gel of the nucleocapsid preparation shown in Fig. 7c and d. The results of limited proteolysis (5) by *S. aureus* protease V8 (Fig. 8) indicated that all the small bands shared the peptide pattern of NP.

ii. Membrane components. The material sedimenting at the CsCl–5% sucrose interface (arrow in Fig. 6) was pelleted and studied by SDS-PAGE. The polypeptide composition of this cytoplasmic fraction is compared with the polypeptides of pure virus and uninfected cells in Fig. 9. The viral polypeptides associated with this fraction are G, F_1, and M (Fig. 9a). In

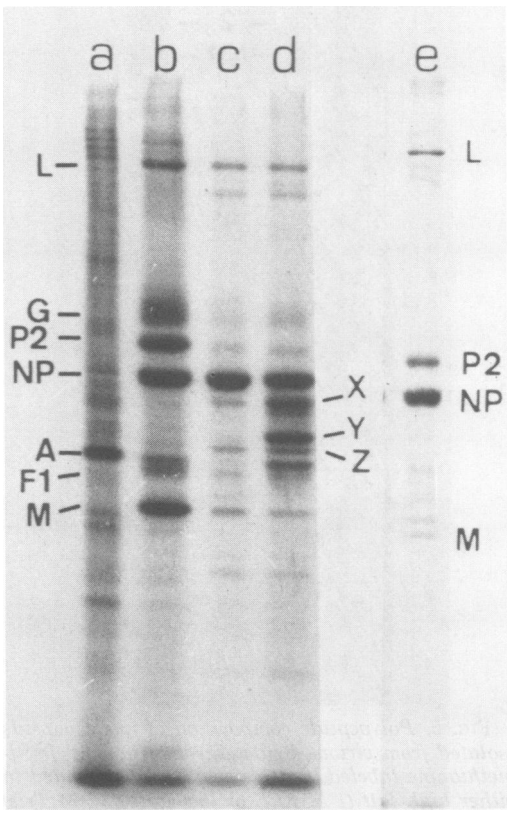

FIG. 7. *[^{35}S]methionine-labeled polypeptides of cytoplasmic nucleocapsids isolated from infected cells. A sample of the nucleocapsid peak from the CsCl gradient shown in Fig. 6 (fractions 9 + 10, ρ ≃ 1.3) was diluted with PBS and pelleted at 34,000 rpm for 90 min in an SW41 rotor. A second sample of the same nucleocapsid preparation was rebanded. The resulting band (ρ ≃ 1.3) was pelleted. Both pellets were processed for SDS-PAGE and autoradiography. Uninfected cytoplasmic extracts and purified virions included as markers were prepared as described in the text. (a) Uninfected cytoplasm; (b) purified virions; (c) nucleocapsids purified by a single CsCl gradient; (d) nucleocapsids isolated after purification on a second CsCl gradient. Electrophoresis was for 2.5 h. (e) Another preparation of cytoplasmic nucleocapsid, ρ ≃ 1.3, isolated on a single CsCl gradient as described above. Electrophoresis was for 2.75 h.*

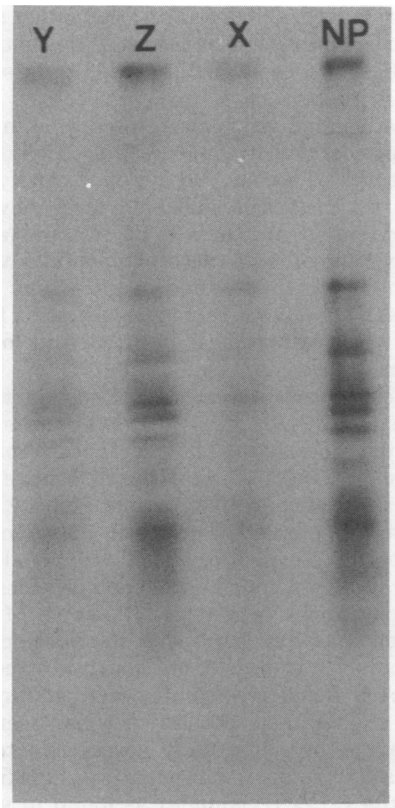

FIG. 8. *Partial protease digests of polypeptides y, z, x, and NP. Bands were cut from preparative SDS-PAGE gels and treated in the manner of Cleveland et al. (5). Digestion was for 30 min with 3 μg of S. aureus protease V8 per sample slot.*

addition, a minor band is found in the position of NP, which may be (i) NP polypeptide that has remained associated with membrane components throughout extraction; (ii) a cellular membrane protein; or (iii) residual F_0 polypeptide that escaped reducing conditions and migrated as a disulfide-bonded 60,000-dalton polypeptide. A surprising result is the absence of actin in the membrane preparation. The G polypeptide seen in the membrane preparation has the fuzzy appearance common to the G band obtained from purified virions, but appears to migrate somewhat faster than virion G (Fig. 9a and b). In addition, the membrane-associated G (Fig. 9a) has a different appearance than the G from Nonidet P-40 cytoplasms (Fig. 1c). These differences may well indicate various states of glycosylation.

DISCUSSION

The growth of measles virus at 33°C rather than 37°C resulted in the release of increased

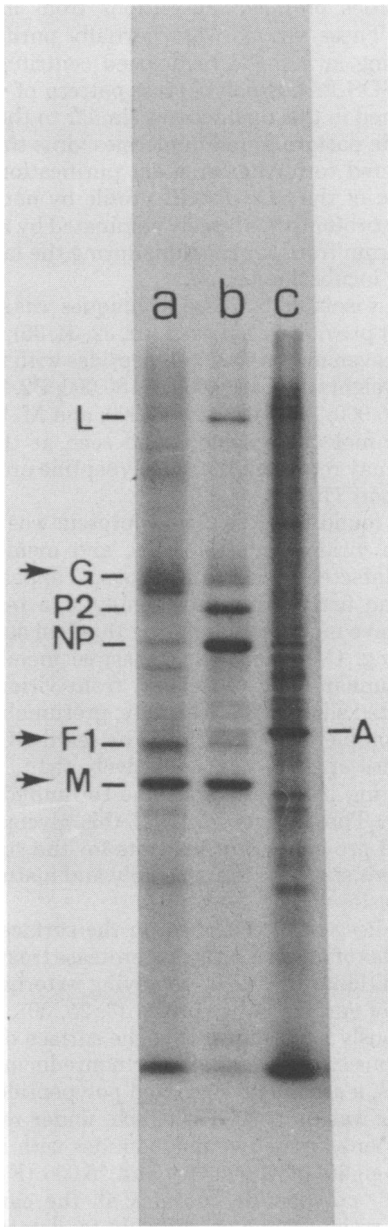

FIG. 9. *Analysis of membrane fraction isolated from the CsCl-sucrose interface of cells infected with measles virus. Fractions 18 and 19 from the CsCl-sucrose gradient shown in Fig. 6 were pooled, diluted with PBS, and pelleted at 34,000 rpm for 90 min in a Beckman SW41 rotor. The isolated pellets were processed for SDS-PAGE and autoradiography as described in the text. (Lane a) 34,000-rpm pellet of fractions 18 and 19; (b) 24,000-rpm pellet of virions; (c) [^{35}S]methionine-labeled uninfected cytoplasm. Electrophoresis was for 2.5 h.*

quantities of infectious virions from infected cells. These virions were partially purified by pelleting in a single high-speed centrifugation. The SDS-PAGE polypeptide pattern of virions obtained in this manner was similar to the polypeptide pattern found in measles virus that was subjected to further gradient purification. Distortion of the SDS-PAGE profile by nonradioactive proteins was largely eliminated by removing serum from the medium during the last half of the incubation period.

Virus isolated by these techniques was similar to that previously reported (22, 32, 34, 36). There were seven major viral polypeptides with molecular weights of: L, 200,000; G, 80,000; P2, 70,000; NP, 60,000; A, 43,000; F_1, 41,000; and M, 37,000. A low-molecular-weight band seen at the dye front may represent the F_2 polypeptide proposed by others (10, 14, 29, 32).

We found that the G glycoprotein was different in virions, infected cells, and membranes from infected cells. From virions, G appeared as a broad fuzzy band, presumably as a result of extensive modification during the final stages of budding. G found on cytoplasmic membranes was similar to that isolated from virions but migrated slightly more rapidly, presumably due to incomplete modification. The cellular G polypeptides appeared as a relatively tight, faster-migrating band, probably due to limited processing. The various forms of this glycoprotein should provide useful reagents for the study of different stages in the assembly and maturation of measles virus.

Studies were performed on the surface polypeptides of measles, utilizing protease treatment, a traditional means of removing external portions of surface polypeptides (3, 25, 30). It had previously been shown that the surface of measles consists of G and, under nonreducing conditions, a second glycosylated polypeptide (molecular weight $\simeq$ 60,000) which, under reduced conditions, forms two polypeptides with molecular weights of 41,000 (F_1) and 15,000 (F_2) (14). The F_2 polypeptide contains all the carbohydrate residues and is difficult to detect with [^{35}S]methionine label. Norrby and Gollmar (28) have described two distinct antigenic activities for intact virions. The hemagglutinating activity is localized on the G glycoprotein, and F is probably the hemolysin polypeptide. Previous reports have shown that trypsin treatment actually abolishes hemagglutination while enhancing hemolytic activity (28). Since F_1 is resistant to trypsin digestion (32), these results suggest that F_1 is not exposed at the exterior of the virion. We have found that at higher concentrations of trypsin both the G and F_1 polypeptides were removed, demonstrating that under appropriate conditions F_1 is indeed sensitive to trypsin and thus must be partially exposed at the surface of the virion.

The pelleted virions were used to obtain nucleocapsids, which could be isolated as stable structures of density ~1.3 g/cm^3. Analysis of these nucleocapsids indicated that they contained four polypeptides: L, P2, NP, and M. The relative amounts of these polypeptides varied, depending on the method of extraction: under high-salt conditions, there is significantly less M than when extraction is performed in low-salt buffer.

Nucleocapsids were also isolated from the cytoplasm of infected cells. Other laboratories have reported the isolation of nucleocapsids from this source (2, 21, 22, 35), but the data have often been contradictory. Our results identify a nucleocapsid complex of density ~1.3 g/cm^3 that consists of polypeptides L-P2-NP or, alternatively, L-NP and one to three polypeptides (x, y, z) shown to be related to NP. Some M polypeptide is always found with the nucleocapsid.

Proteolytic clevage of nucleocapsid structures has been demonstrated in other paramyxoviruses (23, 24), and measles virus has been postulated to be particularly susceptible to this process (22). This is consistent with our finding that the L-P2-NP complex as found in virions (presumably the intact nucleocapsid) is difficult to isolate from infected cytoplasm. Since nucleocapsids from virions that are exposed to equally harsh extraction do not break down, and since the breakdown products are more homogeneous than would be expected from random cleavage, it seems likely that a cytoplasmic process, probably enzymatic, is responsible for the generation of bands x, y, and z.

The L polypeptide, usually considered a host contaminant, a nonspecific aggregate, or at best a viral polypeptide found in variable amounts and with unknown function, appears to be a stable component of the viral nucleocapsid complex. It is found in association with nucleocapsids from cytoplasm and from mature virions, is not removed under the harsh conditions of high-salt and detergent treatment, and is found with the NP polypeptide even in the absence of the usually more plentiful P2 species. The L polypeptide is probably involved in RNA transcription or replication, analogous to the largest proteins of other paramyxoviruses (1, 6).

The smallest virion polypeptide (molecular weight $\simeq$ 37,000) has been called M ("matrix") and by analogy has been assumed to serve the same function as the smallest protein of other paramyxoviruses (22, 32, 36). The M polypeptide

is nonglycosylated, is insensitive to trypsin, but is solubilized by Triton X-100 in the presence of 1 M KCl. The solubility of the M polypeptide in Triton X-100 with high salt suggests that it is a hydrophobic protein. There appears to be strong associations between the M polypeptide and the nucleocapsid structure, since we, as well as others (E. Norrby, personal communication), have been unable to obtain nucleocapsid complexes that are completely devoid of the M polypeptide. The interaction of M with both membranes and nucleocapsid complexes suggests that this polypeptide serves the matrix function described for other enveloped viruses (19, 26, 31, 39).

The association of the M polypeptides and the nucleocapsids of several viruses has been extensively studied. M has been implicated in regulation of viral transcription in Sendai and vesicular stomatitis viruses (5a, 20), and it is thought that one of the major functions of matrix proteins is the collation of viral nucleocapsid and membrane elements during budding (26, 31, 39). Considerable attention has recently been focused on the M polypeptide of measles and its function in viral replication. Comparison of the polypeptides of wild type measles and various subacute sclerosing panencephalitis and persistent viruses indicates that viruses associated with persistent states have an M polypeptide that is electrophoretically distinct from the M of wild-type measles (11, 37, 38; S. L. Wechsler et al., unpublished data). Our ability to isolate fractions enriched in this polypeptide from both virions and infected cells should facilitate study of the possible role of M in persistent infections.

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